

CLAIMS

Sub B1 1. A cell which has been isolated from a living tissue or umbilical blood, and which has the potential to differentiate into at least a cardiomyocyte.

2. The cell according to claim 1, wherein the living tissue is bone marrow.

3. The cell according to claim 1 or 2, wherein the cell is a multipotential stem cell.

4. The cell according to any one of claims 1 to 3, wherein the cell is a multipotential stem cell which differentiates into at least a cardiomyocyte and a vascular endothelial cell. *CB*

5. The cell according to any one of claims 1 to 4, wherein the cell is a multipotential stem cell which differentiates into at least a cardiomyocyte, an adipocyte, a skeletal muscle cell, an osteoblast, and a vascular endothelial cell.

Sub B1 6. The cell according to any one of claims 1 to 5, wherein the cell is a multipotential stem cell which differentiates into at least a cardiomyocyte, an adipocyte,

a skeletal muscle cell, an osteoblast, a vascular endothelial cell, a nervous cell, and a hepatic cell.

7. The cell according to any one of claims 1 to 3, wherein the cell is a multipotential stem cell which differentiates into any cell in adult tissues.

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8. The cell according to any one of claims 1 to 7, wherein the cell is CD117-positive and CD140-positive.

9. The cell according to claim 8, wherein the cell is further CD34-positive.

10. The cell according to claim 9, wherein the cell is further CD144-positive.

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11. The cell according to claim 9, wherein the cell is further CD140-negative.

12. The cell according to claim 8, wherein the cell is CD34-negative.

13. The cell according to claim 12, wherein the cell is further CD144-positive.

14. The cell according to claim 12, wherein the cell is further CD144-negative.

15. The cell according to claim 10, wherein the cell is further CD14-negative, CD45-negative, CD90-negative, Flk-1-negative, CD31-negative, CD105-negative, CD49b-negative, CD49d-negative, CD29-positive, CD54-negative, CD102-negative, CD106-negative, and CD44-positive.

16. The cell according to claim 11, wherein the cell is further CD14-negative, CD45-negative, CD90-negative, Flk-1-negative, CD31-negative, CD105-negative, CD49b-negative, CD49d-negative, CD29-positive, CD54-negative, CD102-negative, CD106-negative, and CD44-positive.

17. The cell according to claim 12, wherein the cell is further CD14-negative, CD45-negative, CD90-negative, Flk-1-negative, CD31-negative, CD105-negative, CD49b-negative, CD49d-negative, CD29-positive, CD54-negative, CD102-negative, CD106-negative, and CD44-positive.

18. The cell according to claim 13, wherein the cell is further CD14-negative, CD45-negative, CD90-negative, Flk-1-negative, CD31-negative, CD105-negative, CD49b-negative, CD49d-negative, CD29-positive, CD54-negative, CD102-negative, CD106-negative, and CD44-positive.

19. The cell according to claim 1, which does not take up Hoechst 33342.

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20. A cardiomyocyte precursor which differentiates into only cardiomyocyte induced from the cell according to any one of claims 1 to 19.

21. The cell according to any one of claims 1 to 20, which has the potential to differentiate into a ventricular cardiac muscle cell.

22. The cell according to any one of claims 1 to 20, which has the potential to differentiate into a sinus node cell.

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23. The cell according to any one of claims 1 to 20, wherein the vital tissue or umbilical blood is derived from a mammal.

24. The cell according to claim 23, wherein the mammal is selected from the group consisting of a mouse, a rat, a guinea pig, a hamster, a rabbit, a cat, a dog, a sheep, a swine, cattle, a goat and a human.

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25. The cell according to any one of claims 1 to 8, which is mouse bone marrow-derived multipotential stem cell BMSC (FERM BP-7043).

26. The cell according to any one of claims 1 to 25, which has the potential to differentiate into a cardiomyocyte by demethylation of a chromosomal DNA of the cell.

27. The cell according to claim 26, wherein the demethylation is carried out by at least one selected from the group consisting of demethylase, 5-azacytidine, and dimethyl sulfoxide, DMSO.

28. The cell according to claim 27, wherein the demethylase comprises the amino acid sequence represented by SEQ ID NO:1.

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29. The cell according to any one of claims 1 to 28, wherein the differentiation is accelerated by a factor which is expressed in a cardiogenesis region of a fetus or a factor which acts on differentiation into a cardiomyocyte in a cardiogenesis stage of a fetus.

30. The cell according to claim 29, wherein the factor which is expressed in a cardiogenesis region of a

fetus or the factor which acts on differentiation into a cardiomyocyte in a cardiogenesis stage of a fetus is at least one selected from the group consisting of a cytokine, an adhesion molecule, a vitamin, a transcription factor, and an extracellular matrix.

31. The cell according to claim 30, wherein the cytokine is at least one selected from the group consisting of a platelet-derived growth factor, PDGF; a fibroblast growth factor-8, FGF-8; an endothelin 1, ET1; a midkine; and a bone morphogenetic factor, BMP-4.

32. The cell according to claim 31, wherein the PDGF, FGF-8, ET1, midkine, and BMP-4 comprise the amino acid sequence represented by SEQ ID NO:3 or 5, the amino acid sequence represented by SEQ ID NO:64, the amino acid sequence represented by SEQ ID NO:66, the amino acid sequence represented by SEQ ID NO:68, and the amino acid sequence represented by SEQ ID NO:70, respectively.

33. The cell according to claim 30, wherein the adhesion molecule is at least one selected from the group consisting of a gelatin, a laminin, a collagen, and a fibronectin.

34. The cell according to claim 30, wherein the vitamin is retinoic acid.

35. The cell according to claim 30, wherein the transcription factor is at least one selected from the group consisting of Nkx2.5/Csx, GATA4, MEF-2A, MEF-2B, MEF-2C, MEF-2D, dHAND, eHAND, TEF-1, TEF-3, TEF-5, and MesP1.

36. The cell according to claim 35, wherein the Nkx2.5/Csx, GATA4, MEF-2A, MEF-2B, MEF-2C, MEF-2D, dHAND, eHAND, TEF-1, TEF-3, TEF-5, and MesP1 comprise the amino acid sequence represented by SEQ ID NO:9, the amino acid sequence represented by SEQ ID NO:11, the amino acid sequence represented by SEQ ID NO:13, the amino acid sequence represented by SEQ ID NO:15, the amino acid sequence represented by SEQ ID NO:17, the amino acid sequence represented by SEQ ID NO:19, the amino acid sequence represented by SEQ ID NO:21, the amino acid sequence represented by SEQ ID NO:23, the amino acid sequence represented by SEQ ID NO:25, the amino acid sequence represented by SEQ ID NO:27, the amino acid sequence represented by SEQ ID NO:29, and the amino acid sequence represented by SEQ ID NO:62, respectively.

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37. The cell according to claim 30, wherein the extracellular matrix is an extracellular matrix derived from a cardiomyocyte.

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38. The cell according to any one of claims 1 to 28, wherein the differentiation is inhibited by a fibroblast growth factor-2, FGF-2.

39. The cell according to claim 38, wherein the FGF-2 comprises the amino acid sequence represented by SEQ ID NO:7 or 8.

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40. The cell according to any one of claims 1 to 28, which is capable of differentiating into a cardiomyocyte or a blood vessel by transplantation into a heart.

41. The cell according to any one of claims 1 to 19, which is capable of differentiating into a cardiac muscle by transplantation into a blastocyst or by co-culturing with a cardiomyocyte.

42. The cell according to any one of claims 1 to 28, which is capable of differentiating into an adipocyte by an activator of a nuclear receptor, PPAR- γ .

43. The cell according to claim 42, wherein the activator is a compound having a thiazolidione skeleton.

44. The cell according to claim 43, wherein the compound is at least one selected from the group consisting of troglitazone, pioglitazone, and rosiglitazone.

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45. The cell according to any one of claims 1 to 28, which is capable of differentiating into a nervous cell by transplantation into a blastocyst or by transplantation into an encephalon or a spinal cord.

46. The cell according to any one of claims 1 to 28, which is capable of differentiating into a hepatic cell by transplantation into a blastocyst or by transplantation into a liver.

47. A method for differentiating the cell according to any one of claims 1 to 28 into a cardiac muscle, comprising using a chromosomal DNA-dimethylating agent.

48. A method for redifferentiating the cell according to claim 9 into the cell according to 12, comprising using a chromosomal DNA-dimethylating agent.

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49. A method for redifferentiating a cell which is CD117-negative and CD140-positive into the cell according to claim 8, comprising using a chromosomal DNA-dimethylating agent.

50. The method according to claim 48 or 49, wherein the chromosomal DNA-dimethylating agent is selected from the group consisting of a demethylase, 5-azacytidine, and DMSO.

51. The method according to claim 50, wherein the demethylase comprises the amino acid sequence represented by SEQ ID NO:1.

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52. A method for differentiating the cell according to any one of claims 1 to 28 into a cardiac muscle, comprising using a factor which is expressed in a cardiogenesis region of a fetus or a factor which acts on differentiation into a cardiomyocyte in a cardiogenesis stage of a fetus.

53. The method according to claim 52, wherein the factor which is expressed in a cardiogenesis region of a fetus or the factor which acts on differentiation into a cardiomyocyte in a cardiogenesis stage of a fetus is at least one selected from the group consisting of a cytokine,

an adhesion molecule, a vitamin, a transcription factor, and an extracellular matrix.

54. The method according to claim 53, wherein the cytokine is at least one selected from the group consisting of a platelet-derived growth factor, PDGF; a fibroblast growth factor-8, FGF-8; an endothelin 1, ET1; a midkine; and a bone morphogenetic factor, BMP-4.

sub B) 55. The method according to claim 54, wherein the PDGF, FGF-8, ET1, midkine, and BMP-4 comprise the amino acid sequence represented by SEQ ID NO:3 or 5, the amino acid sequence represented by SEQ ID NO:64, the amino acid sequence represented by SEQ ID NO:66, the amino acid sequence represented by SEQ ID NO:68, and the amino acid sequence represented by SEQ ID NO:70, respectively.

56. The method according to claim 53, wherein the adhesion molecule is at least one selected from the group consisting of a gelatin, a laminin, a collagen, and a fibronectin.

57. The method according to claim 53, wherein the vitamin is retinoic acid.

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58. The method according to claim 53, wherein the transcription factor is at least one selected from the group consisting of Nkx2.5/Csx, GATA4, MEF-2A, MEF-2B, MEF-2C, MEF-2D, dHAND, eHAND, TEF-1, TEF-3, TEF-5, and MesP1.

59. The method according to claim 58, wherein the Nkx2.5/Csx, GATA4, MEF-2A, MEF-2B, MEF-2C, MEF-2D, dHAND, eHAND, TEF-1, TEF-3, TEF-5, and MesP1 comprise the amino acid sequence represented by SEQ ID NO:9, the amino acid sequence represented by SEQ ID NO:11, the amino acid sequence represented by SEQ ID NO:13, the amino acid sequence represented by SEQ ID NO:15, the amino acid sequence represented by SEQ ID NO:17, the amino acid sequence represented by SEQ ID NO:19, the amino acid sequence represented by SEQ ID NO:21, the amino acid sequence represented by SEQ ID NO:23, the amino acid sequence represented by SEQ ID NO:25, the amino acid sequence represented by SEQ ID NO:27, the amino acid sequence represented by SEQ ID NO:29, the amino acid sequence represented by SEQ ID NO:62, respectively.

60. The method according to claim 53, wherein the extracellular matrix is an extracellular matrix derived from a cardiomyocyte.

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61. A method for differentiating the cell according to any one of claims 1 to 28 into an adipocyte, comprising using an activator of a nuclear receptor, PPAR- γ .

62. The method according to claim 61, wherein the activator is a compound having a thiazolidione skeleton.

63. The method according to claim 62, wherein the compound is at least one selected from the group consisting of troglitazone, pioglitazone, and rosiglitazone.

64. A myocardium-forming agent, comprising, as an active ingredient, a chromosomal DNA-demethylating agent.

65. The myocardium-forming agent according to claim 64, wherein the chromosomal DNA-demethylating agent is at least one selected from the group consisting of a demethylase, 5-azacytidine, and DMSO.

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66. The myocardium-forming agent according to claim 65, wherein the demethylase comprises the amino acid sequence represented by SEQ ID NO:1.

67. A myocardium-forming agent, comprising, as an active ingredient, a factor which is expressed in a cardiogenesis region of a fetus or a factor which acts on

differentiation into a cardiomyocyte in a cardiogenesis stage of a fetus.

68. The myocardium-forming agent according to claim 67, wherein the factor which is expressed in a cardiogenesis region of a fetus or the factor which acts on differentiation into a cardiomyocyte in a cardiogenesis stage of a fetus is at least one selected from the group consisting of a cytokine, an adhesion molecule, a vitamin, a transcription factor, and an extracellular matrix.

69. The myocardium-forming agent according to claim 68, wherein the cytokine is at least one selected from the group consisting of a platelet-derived growth factor, PDGF; a fibroblast growth factor-8, FGF-8; an endothelin 1, ET1; a midkine; and a bone morphogenetic factor, BMP-4.

70. The myocardium-forming agent according to claim 69, wherein the PDGF, FGF-8, ET1, midkine, and BMP-4 comprise the amino acid sequence represented by SEQ ID NO:3 or 5, the amino acid sequence represented by SEQ ID NO:64, the amino acid sequence represented by SEQ ID NO:66, the amino acid sequence represented by SEQ ID NO:68, and the amino acid sequence represented by SEQ ID NO:70, respectively.

71. The myocardium-forming agent according to claim 68, wherein the adhesion molecule is selected from the group consisting of a gelatin, a laminin, a collagen, and a fibronectin.

72. The myocardium-forming agent according to claim 71, wherein the vitamin is retinoic acid.

73. The myocardium-forming agent according to claim 68, wherein the transcription factor is at least one selected from the group consisting of Nkx2.5/Csx, GATA4, MEF-2A, MEF-2B, MEF-2C, MEF-2D, dHAND, eHAND, TEF-1, TEF-3, TEF-5, and MesP1.

74. The myocardium-forming agent according to claim 73, wherein the Nkx2.5/Csx, GATA4, MEF-2A, MEF-2B, MEF-2C, MEF-2D, dHAND, eHAND, TEF-1, TEF-3, TEF-5, and MesP1 comprise the amino acid sequence represented by SEQ ID NO:9, the amino acid sequence represented by SEQ ID NO:11, the amino acid sequence represented by SEQ ID NO:13, the amino acid sequence represented by SEQ ID NO:15, the amino acid sequence represented by SEQ ID NO:17, the amino acid sequence represented by SEQ ID NO:19, the amino acid sequence represented by SEQ ID NO:21, the amino acid sequence represented by SEQ ID NO:23, the amino acid

sequence represented by SEQ ID NO:25, the amino acid sequence represented by SEQ ID NO:27, the amino acid sequence represented by SEQ ID NO:29, and the amino acid sequence represented by SEQ ID NO:62, respectively.

75. The myocardium-forming agent according to claim 68, wherein the extracellular matrix is an extracellular matrix derived from a cardiomyocyte.

76. A method for regenerating a heart damaged by a heart disease, comprising using the cell according to any one of claims 1 to 46. *(B)*

77. An agent for cardiac regeneration, comprising, as an active ingredient, the cell according to any one of claims 1 to 46.

sub(B) 78. A method for specifically transfecting a wild-type gene corresponding to a mutant gene in a congenital genetic disease to a myocardium, comprising using the cell according to any one of claims 1 to 46 into which the wild-type gene corresponding to a mutant gene in a congenital genetic disease of a heart has been introduced.

79. A therapeutic agent for a heart disease, comprising, as an active ingredient, the cell according to

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any one of claims 1 to 46 into which a wild-type gene corresponding to a mutant gene in a congenital genetic disease of a heart has been introduced.

80. A method for producing an antibody which specifically recognizes the cell according to any one of claims 1 to 46, comprising using the cell as an antigen.

81. A method for isolating a cell having the potential to differentiate into a cardiomyocyte according to any one of claims 1 to 46, comprising using an antibody obtained by the method according to claim 80.

82. A method for obtaining a surface antigen specific for the cell according to any one of claims 1 to 46, comprising using the cell.

83. A method for screening a factor which proliferates the cell according to any one of claims 1 to 46, comprising using the cell.

84. A method for screening a factor which induces the cell according to any one of claims 1 to 46 to differentiate into a cardiomyocyte, comprising using the cell.

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85. A method for screening a factor which immortalizes the cell according to any one of claims 1 to 46, comprising using the cell.

86. A method for immortalizing the cell according to any one of claims 1 to 46, comprising expressing a telomerase in the cell.

87. The method according to claim 86, wherein the telomerase comprises the amino acid sequence represented by SEQ ID NO:31.

sub B) 88. A therapeutic agent for a heart disease, comprising, as an active ingredient, the cell according to any one of claims 1 to 46 which has been immortalized by expressing a telomerase.

89. The therapeutic agent according to claim 88, wherein the telomerase comprises the amino acid sequence represented by SEQ ID NO:31.

Sub B) 90. A culture supernatant comprising the cell according to any one of claims 1 to 46.

91. A method for inducing the cell according to any one of claims 1 to 46 to differentiate into a

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cardiomyocyte, comprising using the culture supernatant according to claim 90.

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